Crystal Structure at 2.6Å Resolution of Phenylalanyl-tRNA Synthetase Complexed with Phenylalanyl-Adenylate in the Presence of Manganese Ions

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Beamline(s): **X12C**

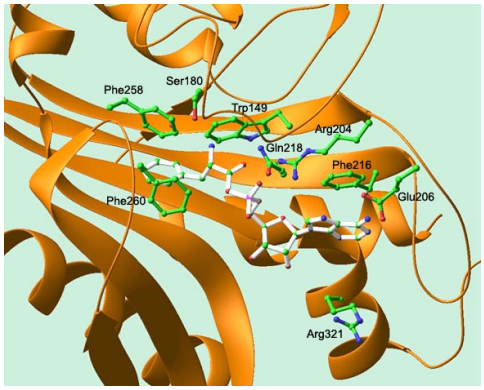

Figure 1. Interactions at the active site. Secondary structure elements are shown that construct class II characteristic active site fold. Phenylalanyl adenylate and side chains of key residues are shown as balls and sticks models. In the case of Phe-AMP interatomic bonds are colored white

Introduction: Aminoacyl-tRNA synthetases (aaRSs) is a family of enzymes, that covalently attach amino acids to a cognate tRNAs through a two-step aminoacylation reaction. At the first step, amino acid is activated by the attack of ATP molecule, giving rise to an intermediate molecule, aminoacyl-adenylate. Based on their structural and functional features, aaRSs are divided into two classes. Phenylalanyl-tRNA synthetase (PheRS) is unique among them, since it have structural properties of class II enzymes, but attaches Phe to 2'-OH group of a ribose of tRNA terminal adenosine, as class I aaRSs do.

Methods and Materials: The crystals of native substrate-free PheRS were soaked with Phe, ATP and Mn^{2+} ions. The data was collected from a single flash-frozen crystal and processed. The model of native PheRS (Mosyak *et al.*, 1995) was used for the refinement. The final model converged at R and R_{free} values of 21.6% and 23.8%, respectively, and demonstrated good stereochemistry.

Results: The crystal structure of Thermus thermophilus PheRS complexed with phenylalanyl-adenylate (Phe-AMP) has been solved at 2.6 angstrom resolution. Specific recognition of phenylalanine portion of PheAMP is achieved by unique mode of aromatic-aromatic interactions. Adenylate moiety of Phe-AMP is recognized in such a manner that all hydrogen bonds, conceivable for adenine ring, both direct and water-mediated, are fully saturated. Divalent metal ion was detected at the α/β subunits interface at a short distance from the active site. This ion is thought to be necessary for an enzyme's activity and stability of $\alpha\beta$ heterodimer.

Conclusions: The determined structure of PheRS complex with a product of a first step of aminoacylation reaction, Phe-AMP, together with previous data, allows to predict a step-by-step mechanism of the highly specific aminoacylation process for phenylalanine system.

References: Mosyak, Reshetnikova, Goldgur, Delarue, Safro, "Structure of phenylalanyl-tRNA synthetase from *Thermus thermophilus*", Nature Structural Biology, **2**, 7, p. 537-547, 1995; Fishman *et al.* (submitted for publication)